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Retention mechanisms of polycyclic aromatic nitrogen heterocyclics on bonded amino phases in normal-phase liquid chromatography

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Abstract

Two aminoalkyl bonded phases, aminopropylsilica and dimenthylaminopropylsilica, operated in the normal-phase mode were investigated regarding the retention mechanisms of polycyclic aromatic nitrogen heterocyclics (PANHs). By blocking active interaction sites with methyl substituents, the retention mechanisms could be ascertained. The bonded functional groups of the aminoalkyl stationary phases were found to be the primary adsorption sites. Hydrogen bonding was found to be the dominant retention mechanism for carbazole-type PANHs on both stationary phases and for acridine-type PANHs on the aminopropyl phase. For acridine-type PANHs on dimethylaminopropylsilica, retention was found to be mainly due to electron donor–acceptor interaction. Residual silanols on the stationary phase support material on these non-end-capped bonded phases were found to contribute only slightly to the retention of both acridine- and carbazole-type PANHs. A strong dependence of retention on steric hindrance of the n-electrons on the nitrogen of acridine-type solutes was shown. Further, the necessity to consider not only the polarity and solvent strength but also the selectivity of the mobile phase is demonstrated.

1. Introduction

Polycyclic aromatic nitrogen heterocyclics (PANHs) with a single endocyclic nitrogen heteroatom, can be divided into two classes: acridines, which contain a pyridine ring with a lone pair electrons on the nitrogen, and carbazoles, containing a pyrrole ring with a hydrogen bonded to the nitrogen atom. Acridine-type PANHs are slightly basic owing to the unshared electron pair of the nitrogen atom not participating in the aromatic delocalization. In carbazole-type PANHs the unshared electron pair is incorporated into the aromatic π orbitals and the imino hydrogen of these compounds gives these PANHs weak acidic properties.

The Snyder–Scozewinski adsorption–displacement model [1,2] has been successfully used in order to explain the retention of polar molecules, including PANHs, on the aminopropylsilica stationary phase [3–5]. In this model, the solute and solvent molecules are expected to compete for positions in a monomolecular layer formed on the surface of the adsorbent. Primarily a monolayer of the solvent is formed on the adsorbent surface. As the solute

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migrates through the column, it competes for the active adsorption sites and retention occurs via solvent displacement–solute adsorption.

However, the specific mechanism of interaction is not considered in the displacement model. Normal bonded phases based on silica are heterogenic materials that contain three forms of surface adsorption sites [6]. They are supposed to consist of the functional groups of the bonded phase, residual silica support silanols and mixed sites as a result of interaction between the functional groups and the residual silanols.

In the aminopropylsilica stationary phase, the adsorbent surface silanol groups are substituted with aminopropyl groups. The surface coverage is about 2 μmol/m^2, compared with 8 μmol/m^2 for the silanol groups of unmodified silica [3]. On the Nucleosil-NH_2 bonded stationary phase, the mean distance between the aminopropyl anchoring sites has been found to be 9.5 Å [7]. The amino group is a strong Lewis base, i.e., an electron donor, and thus shows specific interactions with electron acceptor solutes.

The retention behaviour of PANHs in normal-phase HPLC using bonded stationary phases is dependent on a number of solute–stationary phase interactions. Possible mechanisms are interactions between the stationary phase and (1) the solute aromatic π-electron system [8,9], (2) the lone pair of electrons of the acridine nitrogen heteroatom [8] and (3) the hydrogen atom attached to the carbazole nitrogen heteroatom [10]. In normal-phase chromatography the acid–base properties of the PANHs [8] and steric effects, i.e., the accessibility of the nitrogen atom [8,11], have an impact on retention. Further, if the bonded phase is not end-capped, the unreacted acidic silanol groups on the silica support material may also play a part in the solute–stationary phase interaction [12].

Some different views have been put forward regarding the retention mechanism of PANHs on the aminopropylsilica phase. Some workers have stated that this phase behaves like a deactivated silica [4] and that the residual silanols are of major importance for the retention of PANH [7]. Others have proposed that the primary interaction site of the aminopropylsilica stationary phase is the amino group [3,13]. Solutes containing fused aromatic ring systems, such as polycyclic aromatic hydrocarbons (PAHs) and PANHs, are assumed to interact by a charge-transfer mechanism involving the lone pair of electrons of the aminopropylsilica and the π-electron cloud of the solutes [9]. This is an electron donor–acceptor (EDA) interaction where the amino group of the stationary phase acts as an electron donor and the fused aromatic ring system of the adsorbed solute acts as an electron acceptor [7]. The solute–stationary phase EDA interaction is assumed to be planar. This has been demonstrated by slightly twisted polyphenyls having a weaker retention than flat fused arenes [9].

Few descriptions of the dimethylaminopropylsilica stationary phase have been published. This phase can be regarded as a methyl-substituted aminopropylsilica where the two amino hydrogens of the bonded functional group have been replaced by methyl substituents. Owing to the free electron pair of the amino nitrogen, this stationary phase may also act like an electron donor. It can thus be assumed that the dimethylaminopropylsilica will interact with fused aromatic ring systems with an EDA mechanism similar to that of the aminopropylsilica phase. However, the replacement of the hydrogens of the amino group by methyl groups will exclude some of the possible mechanisms involved in the retention of PANH.

2. Experimental

2.1. Chemicals

Reference substances of acridine-type PANHs (Fig. 1) were purchased from Promochem (Wesel, Germany). The carbazole-type reference substances were kindly provided by Professor M. Zander. Each component was dissolved in pentane–hexane (10:90) (Rathburn, Walkerburn, UK) at a concentration of approximately 10 ng/μl.

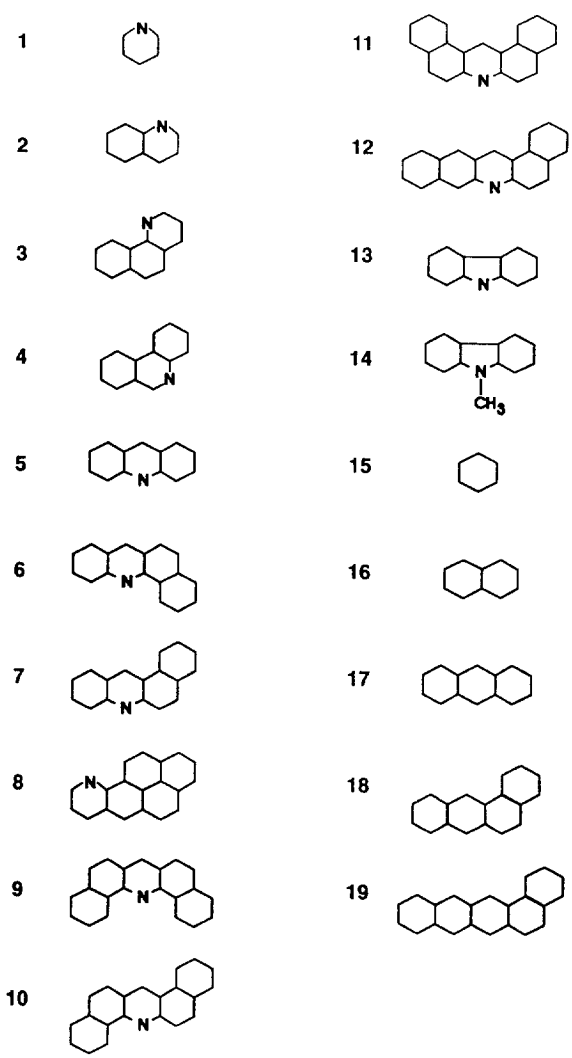

Fig. 1. Structures of the solutes: 1 = pyridine; 2 = quinoline; 3 = benzo[*h*]quinoline; 4 = phenanthridine; 5 = acridine; 6 = benz[*c*]acridine; 7 = benz[*a*]acridine; 8 = 10-azabenzo[*a*]pyrene; 9 = dibenz[*c*,*h*]acridine; 10 = dibenz[*a*,*h*]acridine; 11 = dibenz[*a*,*j*]acridine; 12 = dibenz[*a*,*i*]acridine; 13 = carbazole; 14 = 9-methylcarbazole; 15 = benzene; 16 = naphthalene; 17 = anthracene; 18 = benz[*a*]anthracene; 19 = dibenz[*a*,*i*]-anthracene.

2.2. HPLC columns

Two amino bonded phase columns from Macherey–Nagel (Düren, Germany) were used: aminopropylsilica (Nucleosil-NH_2, 200 × 4 mm I.D.) and dimethylaminopropylsilica [Nucleosil-$N(CH_3)_2$, 200 × 4 mm I.D.). Data on the surface coverage of these two phases were not supplied by the manufacturer. Berendsen et al. [14] calculated the surface coverage for synthesized amino and dimethylamino phases as 2.35 and 3.11 μmol/m^2, respectively. Both of the modified stationary phases were manufactured using Nucleosil 100-5 as the silica base material. This consists of spherical particles with a pore size of 100 Å and a diameter of 5 μm. The pore volume is 1.0 ml/g and the surface area 350 m^2/g. A Nucleosil 100-5 column (200 × 4 mm I.D.) containing the silica base material was also used during the investigation.

2.3. Mobile phases

Hexane, methyl *tert.*-butyl ether (MTBE) and dichloromethane (DCM) were used as mobile phase components. All solvents were of HPLC grade (Rathburn) and were used as received. Regarding the aminopropylsilica and the dimethylaminopropylsilica stationary phases, hexane at a flow-rate of 2.0 ml/min was used during the retention measurements to investigate interaction mechanisms. The retention of carbazole and acridine on the unmodified Nucleosil silica base material was measured using a mobile phase composition of DCM–hexane (40:60). When investigating the influence of steric hindrance of adjacent benzene rings, a mobile phase consisting of MTBE–hexane (10:90) was used in order to obtain reasonable retention times for the unshielded acridine-type PANHs ($k' > 100$ when using pure hexane). When investigating solvent effects, mobile phase compositions consisting of 0, 10, 20, 33 and 50% of polar modifier, either MTBE or DCM, in hexane were used.

2.4. HPLC instrumentation

The HPLC system consisted of an injector (Model 7125; Rheodyne, Cotati, CA, USA)

equipped with a 20-μl injection loop, an HPLC pump (Model 590 programmable solvent delivery module; Waters, Milford, MA, USA) and a UV detector (Model 655A; Hitachi, Tokyo, Japan) monitoring the effluent at 254 nm. Retention time measurements were performed at 20 ± 2°C. A PC-based laboratory data system (ELDS Pro; Chromatography Data Systems, Svartsjö, Sweden) was used for registering, storing and processing the detector signals. Prior to investigation, each column was allowed to equilibrate overnight using a flow of 0.2 ml/min of the mobile phase. The system dead time (t_0) was determined as the first baseline disturbance due to the elution of pentane. Retention time (t_r) was measured as the peak maximum calculated by applying a polynomial function to the obtained data points collected at a sampling rate of 5 Hz. The reported capacity factors (k'), calculated as $k' = (t_r - t_0)/t_0$, where t_0 is the dead time, are the means of five replicate determinations. For all k' values the relative standard deviation was <4% and mostly <3%, except for acridine on the aminopropylsilica using hexane as mobile phase.

3. Results and discussion

The two aminoalkyl stationary phases were manufactured using the same silica base material and had similar surface coverages of the bonded phase. By studying the retention properties on these two related phases it was possible to determine some of the retention mechanisms of PANHs in more detail. It should be noted that the investigation was made using Nucleosil silica-based packings and the results may differ when using other packings based on other silicas.

3.1. Interaction mechanisms

PAHs are assumed to interact with the amino stationary phase by an EDA type of interaction mechanism [7]. Owing to the delocalized aromatic π-electrons, the PAH solute acts as an electron acceptor and the amino group as a strong n-electron donor [9]. When plotting log k' (Table 1) versus the number of π-electrons for the five PAHs on the amino and the dimethylamino columns, two straight ($r > 0.999$), parallel (slope 0.193 ± 0.005 and 0.204 ± 0.015,

Table 1
Capacity factors (k') and relative retentions versus anthracene (α) for some selected polycyclic aromatic hydrocarbons and polycyclic aromatic nitrogen heterocyclics on the aminoporpylsilica and the dimethylaminopropylsilica stationary phases with hexane as mobile phase at a flow-rate of 2.0 ml/min

Compound	Stationary phase			
	Aminopropylsilica		Dimethylaminopropylsilica	
	k'	α	k'	α
Benzene	0.39	0.22	0.14	0.18
Naphthalene	0.83	0.46	0.35	0.44
Anthracene	1.80	1	0.80	1
Benz[*a*]anthracene	3.84	2.13	1.63	2.04
Dibenz[*a,i*]anthracene	8.69	4.83	3.82	4.77
Pyridine	58.6	32.5	6.20	7.75
Quinoline	77.5	43.1	6.96	8.70
Acridine	161	89.7	7.55	9.44
Benz[*a*]acridine	>200	>111	11.4	14.2
Dibenz[*a.i*]acridine	>200	>111	21.4	26.8
Carbazole	116	64.6	162	203
9-Methylcarbazole	5.63	3.13	1.97	2.46

respectively, at a confidence level of 95%) lines over a range of log k' of approximately 0.9 were obtained. Thus, both columns demonstrated similar selectivity towards PAHs, but the dimehtylamino phase exhibited weaker retention. It is therefore plausible to assume that the same major retention mechanism, a charge-transfer interaction [7,9], is acting in both cases.

The introduction of the two methyl substituents on the amino group introduces two competing mechanisms, a steric and an inductive effect, that will change the retention when comparing the two stationary phases. Steric effects by the more bulky methyl groups yield a larger distance for the charge-transfer interaction and thus a decrease in retention. On the other hand, an increase in retention would be the result of the inductive effect on addition of methyl groups due to stronger electron donor properties of the nitrogen lone pair of electrons. The observed decrease in retention on the dimethylamino phase, a factor of ca. 2.5, demonstrate that the steric effect of the methyl groups is of greater importance than the increased electron density on the amino nitrogen for this EDA interaction.

Carbazole has three linear fused rings, two six-membered and one five-membered ring, and fourteen electrons participating in the electron delocalization, including the unshared electron pair of the nitrogen heterocyclic atom. The compound possess weak acidic properties due to the hydrogen atom on the pyrrolic ring. Thus, in the case of carbazole-type compounds, three retention mechanisms are possible: (1) charge-transfer interaction as described above; (2) hydrogen bonding interaction between the acidic imino hydrogen attached to the nitrogen heteroatom of the solute and the nitrogen lone pair of electrons of the stationary phase amino group [4]; and (3) hydrogen bonding between the solute imino hydrogen and residual silanols on the adsorbent surface. These interactions are possible on both the aminopropyl- and the dimethylaminopropylsilica stationary phases.

That hydrogen bonding is the major retention mechanism for carbazole-type compounds is obvious from the strong decrease in retention on replacing the imino hydrogen of carbazole with a methyl group (9-methylcarbazole). No hydrogen bonding is then possible and the retention on the aminopropyl phase decreases from $k' = 116$ to 5.63, a factor of >20 (Table 1). A stronger effect was found for the dimethylaminopropylsilica phase, where this factor was >80. That this is due to hydrogen bonding to the bonded amino group of the stationary phase and not residual silanols is obvious from the following: in the next paragraph it is clearly demonstrated that the basic solute acridine has only a minor interaction with the residual silanols of the acidic silica surface. The retention of carbazole on the unsubstituted silica base material using DCM–hexane (40:60) was found to be >100 times weaker than for acridine. It is therefore obvious that carbazole has to exhibit an even weaker interaction with residual silanols on these two bonded amino stationary phases. Further, the introduction of methyl substituents on the amino group of the stationary phase increased the retention of carbazole from $k' = 116$ to 162, a factor of 1.4. This is attributed to the inductive effect of the methyl groups on the stationary phase that increase the electron density of the amino nitrogen, resulting in a stronger hydrogen bonding interaction. 9-Methylcarbazole exhibits a stronger retention than anthracene (5.63 and 1.80, respectively) on the aminopropyl column. As the polar interaction site of carbazole is blocked by the methyl substituent, the increased retention is attributed to the inductive effect of the heterocyclic nitrogen of the solute increasing the electron acceptor ability of the π-electron system, thus yielding stronger EDA complex formation than for anthracene.

Acridine has a similar structure to anthracene. Both compounds consist of three linear fused-six membered rings, with fourteen π-electrons involved in the electron delocalization. On the aminopropylsilica the k' value for acridine was found to be 161 compared with 1.80 for anthracene (Table 1). This increased retention can be attributed to (1) an increased charge-transfer interaction with stronger EDA complex formation involving the delocalized π-electrons [7], (2)

a hydrogen bonding interaction between the weakly basic solute nitrogen lone pair of electrons and the amino hydrogens of the stationary phase [4] and/or (3) hydrogen bonding to residual silanols of the adsorbent surface. On the dimethylaminopropylsilica phase the possibility of hydrogen bonding with the amino group was eliminated by replacing the amino hydrogens with methyl groups. From the strong decrease in the retention of acridine, from $k' = 161$ to 7.55, it is clear that the major retention mechanism does not involve hydrogen bonding to the residual silanols as stated by Hammers et al. [7].

That the EDA interaction is a minor retention mechanism for PANHs is evident from the retentions of anthracene, carbazole and 9-methylcarbazole, $k' = 1.80$, 116 and 5.63, respectively, on the aminopropylsilica phase. In the last two compounds the n-electrons of the heterocyclic nitrogen participate in the electron delocalization. Further, the hydrogen bonding site, the carbazole imino hydrogen, is blocked by a methyl substituent in 9-methylcarbazole. No hydrogen bonding is therefore possible between the solute nitrogen lone pair of electrons and the amino hydrogens of the aminopropylsilica stationary phase. Hence, the only remaining retention mechanism is EDA complex formation. 9-Methylcarbazole has a retention approximately three times stronger than that of anthracene owing to the inductive effect of the nitrogen atom on the electron-accepting properties of the delocalized electrons. The retention of carbazole is, however, >20 times stronger than that of 9-methylcarbazole, clearly demonstrating a stronger interaction due to hydrogen bonding.

The steric effect on the retention of anthracene and 9-methylcarbazole by the methyl groups of the dimethylaminosilica is a decrease by a factor of about 2–3 while the decrease in the retention of acridine is a factor of about 21. The obvious reason for this 7–10 times stronger decrease is the blocking of the hydrogen bonding to the bonded functional amino group of the adsorbent. The stronger retention of acridine compared with anthracene on the dimethylaminopropylsilica is attributed, in analogy with 9-methylcarbazole, to increased EDA complex formation due to the inductive effect of the heterocyclic nitrogen atom.

With an increasing number of aromatic rings attached to the pyridine or pyrrole rings, the relative contribution of hydrogen bonding will decrease. This is demonstrated in Fig. 2 by the non-linear plot of log k' obtained from the dimethylaminopropylsilica phase vs. number of π-electrons for five aromatic compounds with 1–5 aromatic rings containing one pyridine ring in the structure, i.e., pyridine, quinoline, acridine, benz[*a*]acridine and dibenz[*a*,*i*]acridine. The five corresponding unsubstituted PAHs demonstrate a linear dependence ($r > 0.999$) on the number of π-electrons. Regarding the pyridine-type compounds, the slope of the line approaches closer to the slope for the PAHs as the number of aromatic rings increases. This behaviour demonstrates that the retention mechanism of pyridine-type compounds consists of more than one interaction. One is the EDA complexation, which is a linear function of the number of aromatic rings, as for the PAHs, and another interaction has a decreasing influence on retention as the number of rings increases. The

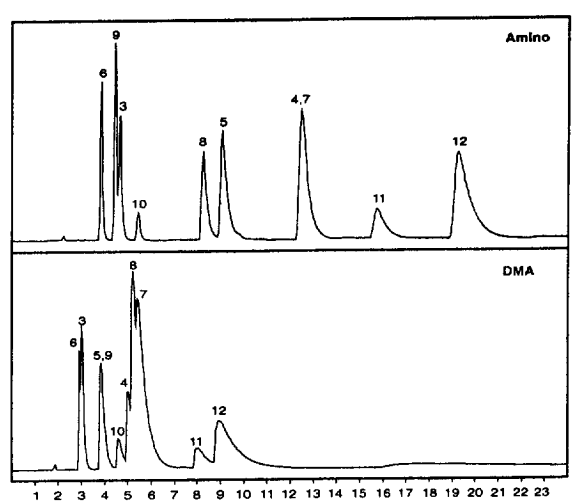

Fig. 2. Chromatograms of a mixture of the acridine-type PANHs listed in Table 2. The peak numbering correspond to the numbers in Fig. 1. Amino = aminopropylsilica stationary phase; DMA = dimethylaminopropylsilica stationary phase. Mobile phase: methyl *tert.*-butyl ether–hexane (10:90) at a flow-rate of 2.0 ml/min. UV detection at 254 nm.

remaining, possible interaction on this phase is hydrogen bonding to residual silanols of the adsorbent surface. It can therefore be concluded that this interaction gives a small contribution to the retention of acridine-type compounds on the dimethylaminopropylsilica and thus also on the aminopropylsilica phase. Hence the residual silanols on the stationary phase support material on these non-end-capped bonded phases can play only a minor role in the retention mechanism for both acridine- and carbazole-type compounds.

3.2. Steric hindrance

There is a shielding effect of the nitrogen atom lone pair of electrons by benzo and methyl groups adjacent to the nitrogen heteroatom of acridine-type PANHs that decreases the strength of the hydrogen bonding interaction with the stationary phase due to steric interaction [8]. This effect was investigated using a mobile phase composition of MTBE–hexane (10:90) in order to obtain reasonable retention times for unshielded acridine compounds with four and five aromatic rings (Table 2). Acridine had $k' = 6.0$ on the aminopropylsilica phase. For the unshielded five-ring compound dibenz[*a*,*i*]acridine, $k' = 13.7$. By shielding the nitrogen lone pair of electrons with one and two adjacent benzo substituents, as in dibenz[*a*,*h*]acridine and dibenz[*c*,*h*]acridine, k' was reduced to 3.2 and 2.4, respectively. For the aminopropylsilica phase, all five investigated PANHs with three to five aromatic rings (benzo[*h*]quinoline, benz[*c*]acridine, 10-azabenzo[*a*]pyrene, dibenz[*a*,*h*]acridine and dibenz[*c*,*h*]acridine) having the nitrogen lone pair of electrons shielded by one or two adjacent benzo substituent groups eluted prior to acridine. Their relative retentions with respect to acridine were in the range 0.3–0.5. This effect is much more pronounced on the aminopropylsilica than the dimethylaminopropylsilica phase. The latter has a corresponding selectivity factor range of 0.7–1.6 for the shielded compounds. This is due to the strong contribution of hydrogen bonding to the retention of acridine type compounds on the former stationary phase. As a decrease in capacity factor due to shielding is observed also for the dimethylaminopropylsilica phase, this implies that the nitrogen lone pair of electrons contribute to the charge-transfer interaction with the stationary phase. Chromatograms obtained on the two stationary phases of a

Table 2
Capacity factors (k') and relative retentions versus acridine (α) for some selected acridine-type PANHs on aminopropylsilica and diamethylaminopropylsilica stationary phases

Compound	Stationary phase			
	Aminopropylsilica		Dimethylaminopropylsilica	
	k'	α	k'	α
Benz[*c*]acridine[a]	1.89	0.31	1.67	0.67
Dibenz[*c*,*h*]acridine[b]	2.37	0.39	2.51	1.00
Benzo[*h*]quinoline[a]	2.61	0.43	1.67	0.67
Dibenz[*a*,*h*]acridine[a]	3.19	0.53	3.30	1.31
10-Azabenzo[*a*]pyrene[a]	5.45	0.53	4.03	1.61
Acridine	6.04	1	2.51	1
Benz[*a*]acridine	8.69	1.44	4.55	1.81
Phenanthridine	8.93	1.48	3.64	1.45
Dibenz[*a*,*j*]acridine	11.2	1.86	6.85	2.72
Dibenz[*a*,*i*]acridine	13.7	2.27	7.87	3.14

Mobile phase: methyl *tert.*-butyl ether–hexane (10:90) at a flow-rate of 2.0 ml/min.
[a] Nitrogen atom shielded by one adjacent benzene ring.
[b] Nitrogen atom shielded by two adjacent benzene rings.

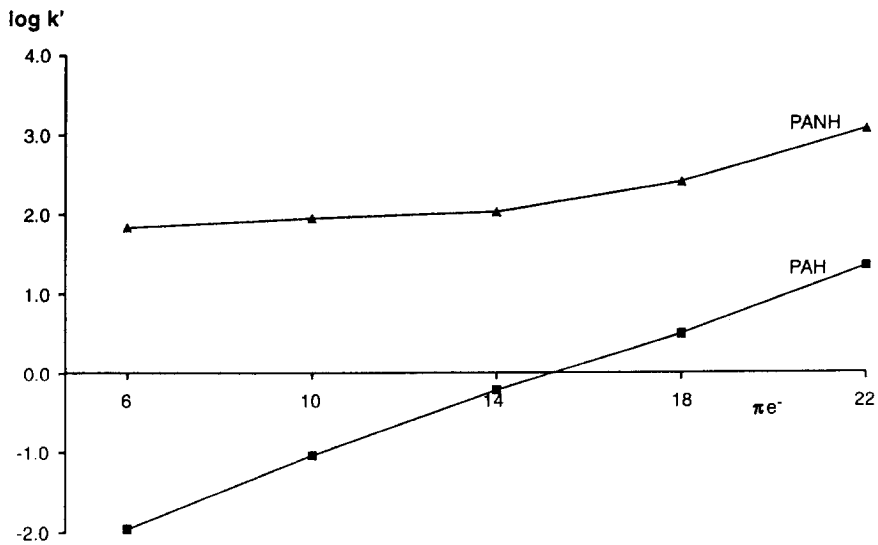

Fig. 3. Log k' as a function of the number of π-electrons for PAHs and acridine-type PANHs with 1–5 aromatic rings. Dimethylaminopropylsilica stationary phase with hexane as mobile phase at a flow-rate of 2.0 ml/min. Mean of five replicate measurements.

mixture of the ten acridine-type PANHs listed in Table 2 are shown in Fig. 3.

3.3. Solvent effects

MTBE and DCM mixed with hexane were used as mobile phases. The concentrations of the polar modifiers in hexane were 0, 10, 20, 33 and 50%. In Fig. 4, log k' for acridine is plotted vs. mobile phase composition for the amino- and dimethylaminopropylsilica phases. On the amino phase the retention of acridine is stronger when using DCM as a polar modifier than when using MTBE, in spite of the higher solvent strength of DCM compared with MTBE, $\varepsilon^0 = 0.42$ and 0.38, respectively. On the dimethylamino phase, on the other hand, the retention of acridine is stronger when using MTBE, which is in accordance with the solvent strength of these two polar modifiers. The addition of a polar mobile phase modifier will increase the solubility of acridine in the mobile phase. If this was the only mechanism, acridine would have a stronger retention on both stationary phases when using MTBE as modifier. However, MTBE is an aprotic, dipolar solvent and a proton acceptor with a slightly basic character. Hydrogen bonding can therefore occur between the MTBE ether oxygen and the amino hydrogens of the aminopropylsilica phase. This is not possible on the dimethylaminopropylsilica phase owing to the methyl groups attached to the amino nitrogen. Hence there is a possibility of adsorption site competition with the solvent when using MTBE as mobile phase modifier on the aminopropylsilica [7]. The conclusion is that the hydrogen bonding interaction of acridine with the amino hydrogens of the aminopropylsilica phase is reduced in strength by adsorption site competition with MTBE. As DCM is unable to interact with the amino hydrogens by hydrogen bonding, retention is influenced only by an increased solubility of acridine in the mobile phase.

Regarding carbazole, the addition of a polar modifier decreases the retention on both columns, which is in accordance to the solvent strength of the two polar solvents, i.e., the addition of DCM gives the shortest retention. As carbazole interacts by hydrogen bonding to the lone pair of electrons on the amino nitrogen, no

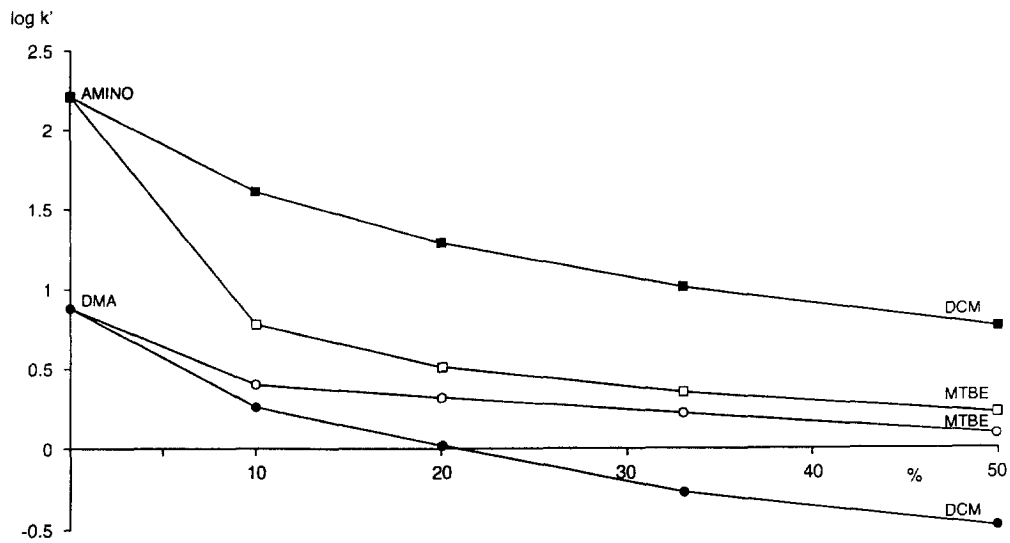

Fig. 4. Log k' for acridine as a function of the percentage of the polar modifiers dichloromethane (DCM) and methyl *tert.*-butyl ether (MTBE) in the hexane mobile phase. Squares = aminopropylsilica stationary phase; circles = dimethylaminopropylsilica stationary phase; closed symbols = DCM; open symbols = MTBE. Mean of five replicate measurements.

adsorption site competition involving the mobile phase can occur regarding the retention of carbazole on either of the two stationary phases.

These results emphasize the importance of hydrogen bonding of the mobile phase to the stationary phase as a contributor to the retention mechanism. It is therefore necessary to consider not only the polarity and solvent strength, but also the selectivity of the mobile phase.

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